Standard Operating Procedure For the Determination of Glyphosate in Water byHigh Performance Liquid Chromatography

1.0 Purpose

1.1 The purpose of this standard operating procedure (SOP) is to allow for qualitative and quantitative determination of the herbicide glyphosate that may be present in water.

2.0 Scope

2.1 The glyphosate analysis is done by utilizing High Performance Liquid Chromatography (HPLC) with post column derivatization. This method is modeled after the Environmental Protection Agency (EPA) Method 547.[1] Glyphosate is separated by the HPLC Ion Exclusion column. It is then oxidized by sodium hypochlorite followed by a reaction with a complex solution to form a derivative that is detected by a fluorescence detector. This SOP is applicable to water samples such as drinking water, ground water, and surface water. The method detection limit is currently 10.0 ug/L.

Glyphosate (Analyte number 30620)

3.0 Safety

3.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Therefore, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. Material safety data (MSD) sheets should be on file for all analytes and reagents.[1] One reagent in particular, 2-mercaptoethanol, has an offensive odor and is considered highly toxic. Special care should be taken when working with this reagent to minimize exposure.

4.0 Interferences

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts and/or elevated baselines in liquid chromatograms. The method must be demonstrated to be free from interferences by running laboratory reagent blanks with each analysis. Two specific sources of interferences are listed below.[1]

4.1.1 Glassware must be scrupulously cleaned. Wash the glassware with water and detergent followed by rinsing with distilled water. Allow the glassware to dry and then rinse with acetone followed by hexane.[1]

4.1.2 The use of high purity reagents and solvents helps to minimize interferences.[1]

5.0 Sample Collection, Preservation, and Handling

5.1 Sample equipment and procedure

5.1.1 Equipment: Samples must be collected in glass bottles with screw caps equipped with a PTFE-face (teflon-face). Prior to use, wash bottles and caps as described in 4.1.1.[1]

5.1.2 Procedure: Conventional sampling practices should be followed.[2]

5.2 Sample Preservative: Samples known or suspected to contain residual chlorine must be preserved with sodium thiosulfate (100 mg/L).[1]

5.3 Sample Storage: Samples must be iced or refrigerated at 4°C away from light. The samples must be analyzed within 14 days of collection.[1]

6.0 Chemicals, Reagents, and Stock Solutions

6.1 Chemicals

6.1.1 Reagent water: For use in the HPLC mobile phase and in the preparation of other reagents. Reagent water is defined as water that is reasonably free of contamination that would prevent the determination of any analyte of interest. Distilled water purified by a Barnstead Nanopure II system is suitable for this procedure. The water from the Barnstead system must be filtered through 0.45 uM filters using mechanical means to remove microparticulates that are detrimental to the HPLC system.

6.1.2 Orthophosphoric acid (H_3PO_4), 85% (w/v): reagent grade

6.1.3 Sodium thiosulfate: ACS grade or better

6.1.4 Sodium borate ($Na_2B_4O_7 \cdot 10H_2O$): reagent grade

6.1.5 Ortho-phthalaldehyde (OPA): available from Pickering or Aldrich (also known as phthalic dicarboxaldehyde)

6.1.6 2-Mercaptoethanol: reagent grade (The compound has an offensive odor and is considered highly toxic.)

6.1.7 Methanol: HPLC grade

6.1.8 Acetonitrile: HPLC grade

6.1.9 Potassium Phosphate Monobasic (KH_2PO_4: reagent grade[3]

6.1.10 Sodium Chloride (NaCl): reagent grade[3]

6.1.11 Sodium Hydroxide (NaOH): reagent grade[3]

6.1.12 Clorox bleach

6.1.13 Concentrated nitric acid: reagent grade

6.2 Reagent Solutions

6.2.1 Sodium thiosulfate solution: Dissolve 100 mg of sodium thiosulfate in 1 L of filtered reagent water.[1] This solution will be used to prepare standards and the laboratory blank spike.

6.2.2 Mobile phase: 0.05% H_3PO_4 in reagent water prepared by adding 1 ml H_3PO_4 to 2 L of filtered reagent water and mix well.[3]

6.2.3 Oxidant reagent: Dissolve 1.36 g potassium phosphate monobasic, 11.6 g sodium chloride, and 0.4 g sodium hydroxide in 1 L of reagent water. Stir on a mechanical stirrer until dissolved.[1] This solution must be filtered through a 0.45 uM filter, then add 200 ul of Clorox bleach and mix well.[3]

6.2.4 2-mercaptoethanol (1/1): Mix 10.0 ml of 2-mercaptoethanol and 10 ml of acetonitrile. Do this procedure in a fume hood. Cap tightly and store in a hood.[1]

6.2.5 OPA reagent: Dissolve 19.1 g of sodium borate in 1 L of reagent water. Stir on a mechanical stirrer until dissolved.[1] Dissolve 800 mg of OPA in about 10 ml of methanol. Add to the sodium borate solution. Rinse the OPA container with about 2 ml of methanol and add this to the sodium borate solution also. This solution must be filtered through a 0.45 uM filter, then add 2 ml of 2-mercatoethanol (1/1) and mix well.[3] This solution should be made fresh daily.

6.3 Glyphosate stock solution

6.3.1 Glyphosate standards can be prepared from dry, pure powder or can be purchased from several vendors at certified concentrations. It is recommended to use the certified solutions.

6.3.1.1 Preparation from dry powder: According to EPA Method 547, accurately weigh and dissolve 0.1000 g of pure glyphosate (96% or greater purity) in 1000 ml of reagent water. The concentration of this stock solution will be 1.00 ug/ml.[1]

6.3.1.2 Preparation from commercially prepared solutions: Glyphosate is available from several commercial sources. Prepare dilutions as necessary for the analysis. See section 8.2 for the details on standard preparation.

7.0 Equipment and apparatus

7.1 Analytical equipment

7.1.1 Varian 9010 HPLC pump. The pump can be programmed at the instrument or via computer access. Generally, the initial setup for an analysis is done at the pump itself. The computer takes control when the analysis and data acquisition begins.

7.1.2 TSP AS3000 autosampler. The autosampler is programmed at the instrument only.

7.1.3 TSP FL2000 or FL3000 fluorescence detector.

7.1.4 Pickering PCX 5100 Post-Column Reaction Module

7.2 Miscellaneous apparatus

7.2.1 System for filtering of liquids before use on the HPLC system. This would include a vacuum source, appropriate filtration flasks, and appropriate reservoir for the liquid. The filters must be designed for the filtering of primarily aqueous solutions. The filters are available from Millipore, catalog number HAWP 046 00, pore size 0.45 uM.

7.2.2 Helium gas for the purging and overlaying of the post column reagents to minimize reagent degradation. Helium gas is also used to sparge the mobile phase.

7.2.3 Sample filters: Samples must be filtered to remove particulates before analysis. Filters are available from Whatman (Puradisc 25 TF, 0.45 uM pore size) or Gelman (Acrodisc 3, 0.45 uM pore size).

7.2.4 Disposable syringes to be used when filtering samples.

8.0 Analytical Procedure

8.1 Analytical conditions

8.1.1 HPLC column: Waters IC-Pak Ion-Exclusion; 15 cm X 7.8 mm[3,4]

8.1.2 HPLC guard column: Waters IC-Pak Cartridges[3]

8.1.3 HPLC mobile phase: 0.05% H_3PO_4 - isocratic (See section 6.2.2 for preparation)[3]

8.1.4 Flow: 1.0 ml/min

8.1.5 Method run time: 20 minutes

8.1.6 Data collection time: 20 minutes

8.1.7 Autosampler parameters:

Injection volume: 200 ul
Run time: 18 minutes

All other parameters can remain at default values.

If the mobile phase is significantly changed from the previous analysis, the syringe on the autosampler must be primed with the new mobile phase before use. See the Alcott 738 manual, section 6.4.2 for the procedure.

8.1.8 Fluorescence detector parameters:

Excitation wavelength: 334 nm
Emission wavelength: 450 nm
Rise Time: 5 seconds
Run time: 20 minutes

All other parameters can remain at default values.

8.1.9 Post column reaction system parameters:

Oxidant reagent: See section 6.2.3 for procedure
OPA reagent: See section 6.2.5 for procedure
Flows: Approximately 0.3 ml/min
HPLC column temperature: 55°C
Reaction temperature: 38°C

8.2 Standard preparation

8.2.1 The commercially available glyphosate standard is typically at a concentration of 1000 ug/ml.

8.2.2 Prepare an intermediate standard in reagent water as follows:

Intermediate*:* 1000 ug/ml X 50 ul/5000 ul = 10.0 ug/ml

8.2.3 The analytical standards are prepared in the sodium thiosulfate solution (see section 6.2.1 for preparation) to improve peak shape.

Standard 1: 10.0 ug/ml X 10 ul/10000 ul = 10.0 ng/ml
Standard 2: 10.0 ug/ml X 15 ul/10000 ul = 15.0 ng/ml
Standard 3: 10.0 ug/ml X 25 ul/10000 ul = 25.0 ng/ml
Standard 4: 10.0 ug/ml X 40 ul/10000 ul = 40.0 ng/ml

8.3 Spike preparation

8.3.1 The commercially available glyphosate standard is typically at a concentration of 1000 ug/ml. A stock spike solution is prepared as follows:

Spike stock: 1000 ug/ml X 1000 ul/5000 ul = 200 ug/ml

8.3.2 Prepare an intermediate spike solution in reagent water from the spike stock as follows:

Intermediate: 200 ug/ml X 50 ul/5000 ul = 2.0 ug/ml = 2000 ng/ml

8.3.3 The lab must analyze one lab fortified blank (blank spike) and one fortified sample (sample spike) with every 10 samples or one per sample set, whichever is greater. The lab fortified blank and lab fortified sample is prepared as follows:

Spike: 2000 ng/ml X 50 ul/5000 ul = 20 ng/ml

8.4 Sample Preparation

8.4.1 The samples have a 14 day hold time. The samples are filtered through the HPLC filters mentioned in section 7.2.3.

8.5 HPLC setup and equilibration

8.5.1 The analytical HPLC system must be setup first. Always have the analytical pump operating before turning on the post column flows. The post column system will not function without pressure on the system from the analytical pump. This is a built in safety feature to prevent post column reagents from back flowing into the HPLC column.

8.5.2 Once the analytical pump is operating, prime the post column pump with the reagents. Allow this to equilibrate for 30 minutes.

8.6 HPLC analysis

8.6.1 Transfer standards and samples to autosampler vials. The standards should be injected at the beginning and end of the analysis. If a large number of samples are to be analyzed, standards may be injected in the middle of the analysis. Computer setup and use of software will not be included in this SOP. It will be left to the analyst to determine the procedures as the procedures vary from instrument to instrument.

8.7 Post column shutdown

8.7.1 The analytical pump must always be flowing while the post column system is pumping. The post column system will shut down if there is no back pressure from the analytical pump. This prevents post column reagents from back flowing into the analytical column.

8.7.2 When the analysis is complete, replace the post column reagent with reagent water and pump for about 15 minutes.

8.7.3 Replace the water with a solution of 20% nitric acid. The post column system is very sensitive to reagents plugging filters and causing high reagent pressures. Pickering recommends this step to minimize this effect. Pump the nitric acid solution for about 1 hour. Pump the nitric acid solution through the post column system only. Do not pump through the analytical pump or the detector.

8.7.4 Replace the 20% nitric acid solution with reagent water and pump for about 15 minutes.

8.7.5 Replace the water with 50/50 methanol/water and pump for an extended period of time, preferably overnight. Leave the reaction temperature at 38°C. This will help any deposits present dissolve.

8.7.6 Set the reaction temperature and column temperature to 20°C and allow the system to cool. This will take several hours. The reagent flows can be shut off after the system has cooled slightly. The 50/50 methanol/water can remain in the system while not in use.

9.0 Data analysis

9.1 Identification of glyphosate

9.1.1 Identification of glyphosate is done by comparing retention times of glyphosate in the samples to glyphosate in the standard injections. The retention time of glyphosate under these conditions is typically around 8 minutes. HPLC analytes are subject to retention time shifts. The window of acceptability is usually ±20 seconds of the average standard retention time.

9.1.2 Typical chromatograms are included at the end of this SOP.

10.0 Quality Control

10.1 Quality control consists of the analysis of laboratory reagent blanks, laboratory fortified blanks and laboratory fortified samples.[1]

10.1.1 Laboratory reagent blanks: A laboratory reagent blank (blank) must be analyzed with each set of samples. If within the retention time window of glyphosate the blank produces a peak that would prevent the determination of glyphosate determine the source of the contamination and eliminate the interference before processing samples.[1]

10.1.2 Laboratory fortified blanks: The analyst must analyze at least one laboratory fortified blank (blank spike) with every 10 samples or one per sample set, whichever is greater.[1] The spike should be prepared along with the samples. See section 8.3 for the blank spike preparation procedure.

10.1.3 Laboratory fortified samples: The analyst must analyze at least one laboratory fortified sample (sample spike) with every 10 samples or one per sample set, whichever is greater.[1] The spike should be prepared along with the samples. See section 8.3 for the sample spike preparation.

10.1.4 Control limits and acceptability of results for laboratory fortified blanks (blank spike) and laboratory fortified samples (sample spike) are as follows:

10.1.4.1 Control limits: Control limits are determined by calculating the mean percent recovery (X) and the standard deviation (S) of the percent recovery. The equations for the upper and lower limits are[1]:

Upper limit = X + 3S
Lower limit = X - 3S

10.1.4.2 Acceptability of results: Any glyphosate blank spike or sample spike result whose percent recovery for that set of samples is outside the control limits cannot be reported. The set must be reanalyzed with a new spike or not reported.

10.2 System suitability: No formal system suitability is done on a daily basis. The standards are evaluated as they become available. Generally, if the glyphosate retention time is similar to that of the previous analysis, and the peak shape is good, the system is considered suitable for the analysis. HPLC system pressures are recorded at the beginning of the analysis and compared to previous readings to detect changes that indicate system problems.

11.0 Documentation

11.1 Results are typically recorded in a notebook.

11.2 Compare the quality control samples to their control limits. Record the spike results and note if they pass or fail to indicate result acceptability.

11.3 Computer files: The computer files should be transferred to diskettes and stored for future reference. After the files have been transferred, they can be deleted from the computer hard drive.

11.4 Hard copies of the reports must be filed.

12.0 References

12.1 EPA 547 Determination of Glyphosate in Drinking Water by Direct-Aqueous-Injection HPLC, Post-Column Derivatization, And Fluorescence Detection. (1990)

12.2 ASTM Annual Book of Standards, Part II Volume 11.01, D3370-82, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA 1986

12.3 Environmental Testing & Analysis (Reprint) "Water Watch: Glyphosate in Drinking Water," May/June 1992

12.4 Waters IC-Pak Ion Exclusion Columns Care and Use Manual, Manual Number 10301 Revision 1, August 1989